

## DEVELOPMENTAL MORPHOLOGY OF ASCOMYCETES

XI. *NECTRIA KERA*

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SUMMARY. — Study of the developmental morphology of new species : *Nectria kera* Subramanian & Bhat (Hypocreale), and its anamorph *Cylindrocarpon* sp.

RÉSUMÉ. — Étude de l'organogénèse d'une espèce nouvelle d'Hypocréales : *Nectria kera* Subramanian & Bhat, et de son anamorphe *Cylindrocarpon* sp.

MOTS CLÉS : Ascomycète, Hypocréale, organogénèse, *Nectria*.

This paper is the eleventh in a series on the developmental morphology of Ascomycetes and deals with *Nectria kera* sp. nov. Our observations are based on a study of a fungus isolated from spathe of *Cocos nucifera* L., collected at Kandy, Sri Lanka, and it belongs to the *Coccinea*-group (BOOTH, 1959) of the genus *Nectria*; it differs from other known species of the genus to warrant placement in a new taxon. Single ascospore isolates when inoculated on sterilized pieces of *Cocos nucifera* and incubated in Roux-tubes mature perithecia developed after six weeks. For studying the various stages in the development of the anamorph and teleomorph, methods described earlier (SUBRAMANIAN & BHAT, 1978) were followed.

*Nectria kera* sp. nov. (Fig. 24-27; Plate II, d-h)

*Perithecia superficialia, solitaria vel 2-8 aggregata, globosa ad pyriformia, rubra vel latericia, papillata, ostiolata, 290-520(390) x 220-410(320) μm, stromati pseudoparenchymati debilique insedentia, leavitunicata, sicca intacta. Paries peritheciale, sectione longitudinale, 20-45 μm latum, constanter duobus fere distinctis stratis : stratum externum et stratum internum. Stratum externum 15-25 μm latum, cellulis 2-3 seriebus, crassitunicates, oblongis ad globosis, 10.3-14.5 x 11.2 μm constantibus; stratum vero internum 10-25 μm latum,*

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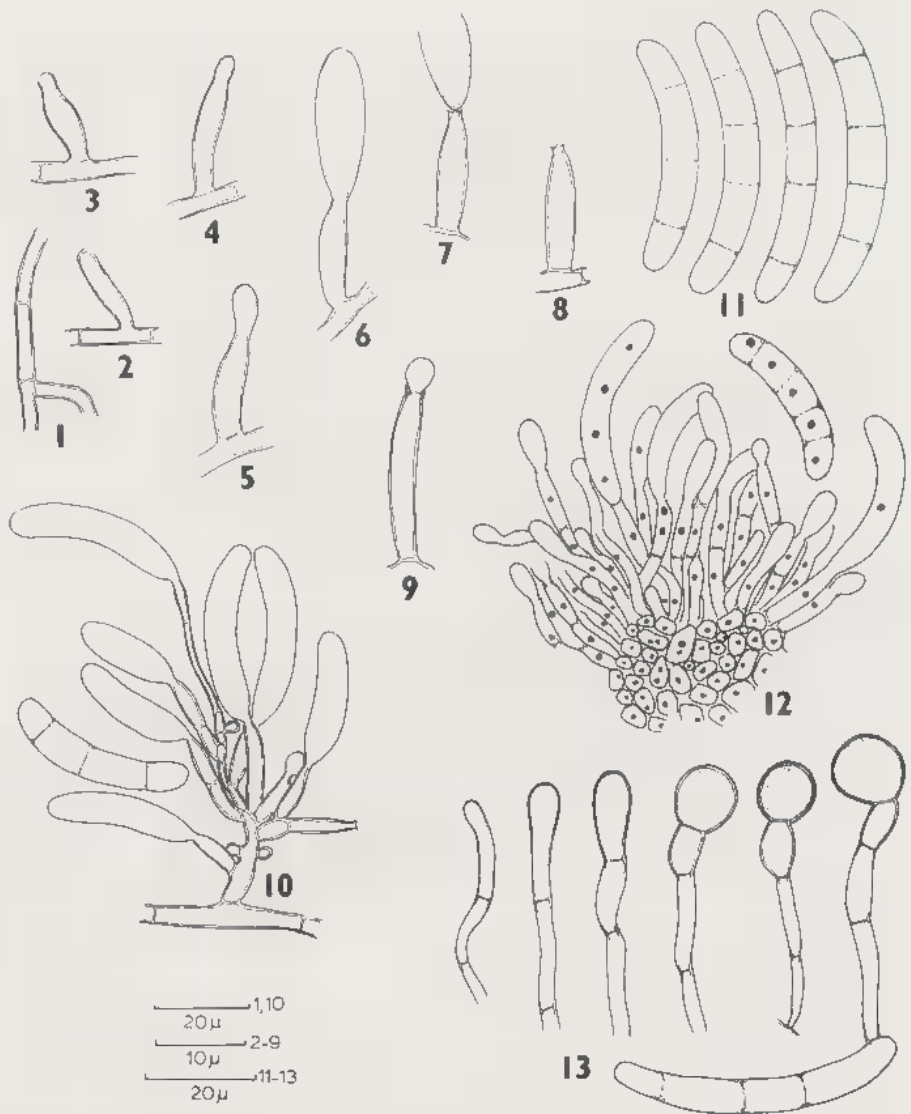


Fig. 1-13 : *Nectria kera*. — 1. portion of a vegetative hypha; 2-9. stages in the development of phialide and conidium; 10. conidiophore bearing phialides and conidia; 11. conidia; 12. section through a sporodochium; 13. stages in the development of chlamydospore.

Fig. 1-13 : *Nectria kera*. — 1. fragment d'hyphe végétative; 2-9. développement d'une phialide et d'une conidie; 10. conidiophore portant des phialides et des conidies; 11. conidies; 12. coupe à travers une sporodochie; 13. développement d'une chlamydospore.

cellulis 5.6-14.0 x 3.2-4.2  $\mu\text{m}$ , tenuitunicatis, angustis, elongatis constantibus; papillae peritheciales breves atque hyphis non ramosis, crassis, parallele dispositis, 50-70  $\mu\text{m}$  altae; 90-100  $\mu\text{m}$  latae.

Asci unitunicate, clavati, brevistipitati, tenuitunicati, 8-sporati, 60-85(68.5) x 10.0-14.0  $\mu\text{m}$ , apicibus rotundatibus atque simplicibus. Ascospores ellipsoideae, 1-septate, aequae bicellulatae, ad septa leaviter constrictae, hyalinae, manifeste verrucosae, 18.0-24.5(22.5) x 6.5-9.0(8.2)  $\mu\text{m}$ ; supra biseriatum, infra vero uniseriatum dispositae in ascus.

Typus : In *Spathie Cocos nucifera* L., collectis Kandy, Sri Lanka, 25-10-1975 a C. V. Subramanian et positus in MUBL Herbario sub numero 2401.

Status conidialis : *Cylindrocarpon* sp.

### CULTURAL CHARACTERS

Ascospores germinating overnight on potato dextrose agar, malt extract agar, and in distilled water producing one or two germ tubes from each cell. Colony on potato dextrose agar pale, attaining a diam. of 2.5-3.0 cm in 12 days, with even margin, with reverse light brown; colony on malt extract agar floccose, dense, attaining a diam. of 3.5-4.5 cm in 12 days, with uneven margin, with surface yellow and reverse purple. On potato dextrose agar mycelium white initially, later becoming pale brown; aerial hyphae septate, branched, up to 2.0-4.2  $\mu\text{m}$  wide (Fig. 1); in old cultures adpressed mycelium becoming thick-walled, pigmented, and with elongated swollen cells 14.0-26.0  $\mu\text{m}$  wide. Conidiophores developing on aerial hyphae, 20-35  $\mu\text{m}$  long, branched or unbranched, septate, with dense cytoplasm, producing phialides laterally and terminally (Fig. 10; Plate I, a). Phialides cylindrical to subcylindrical, slightly swollen at the base, narrowed towards the tip, with a distinct collarette, 12.5-17.5 x 3.0-4.0  $\mu\text{m}$  (Fig. 8), producing only macroconidia.

Macroconidia of nearly uniform width throughout, curved, distinctly dorso-ventral, with smoothly rounded ends, without foot-cell, hyaline, 3-5 septate (Fig. 11; Plate I, g), measuring

3-septate conidia . . . . .	30.0-42.5(37.5) x 5.2-5.6 $\mu\text{m}$
4-septate conidia . . . . .	35.5-45.5(42.5) x 5.5-6.0 $\mu\text{m}$
5-septate conidia . . . . .	50.0-65.5(58.5) x 5.8-6.2 $\mu\text{m}$

Chlamydospores (Fig. 13) terminal on vegetative hyphae, intercalary in conidia, subglobose to globose, 13.5-15.5  $\mu\text{m}$  in diam.

In old cultures, phialides aggregating on plectenchyma composed of thick-walled hyphae and forming sporodochia (Fig. 12); sporodochia 50-90  $\mu\text{m}$  high, 170-210  $\mu\text{m}$  wide, surmounted by cream coloured macroconidia.

The noteworthy features of this fungus are the possession of red and semi-translucent perithecia, perithecial wall being pseudoparenchymatous and composed of irregularly arranged cells of variable shape and size (not filiform or elongated as in *Nectria mammoidea*) in the outer region, large ascospores with

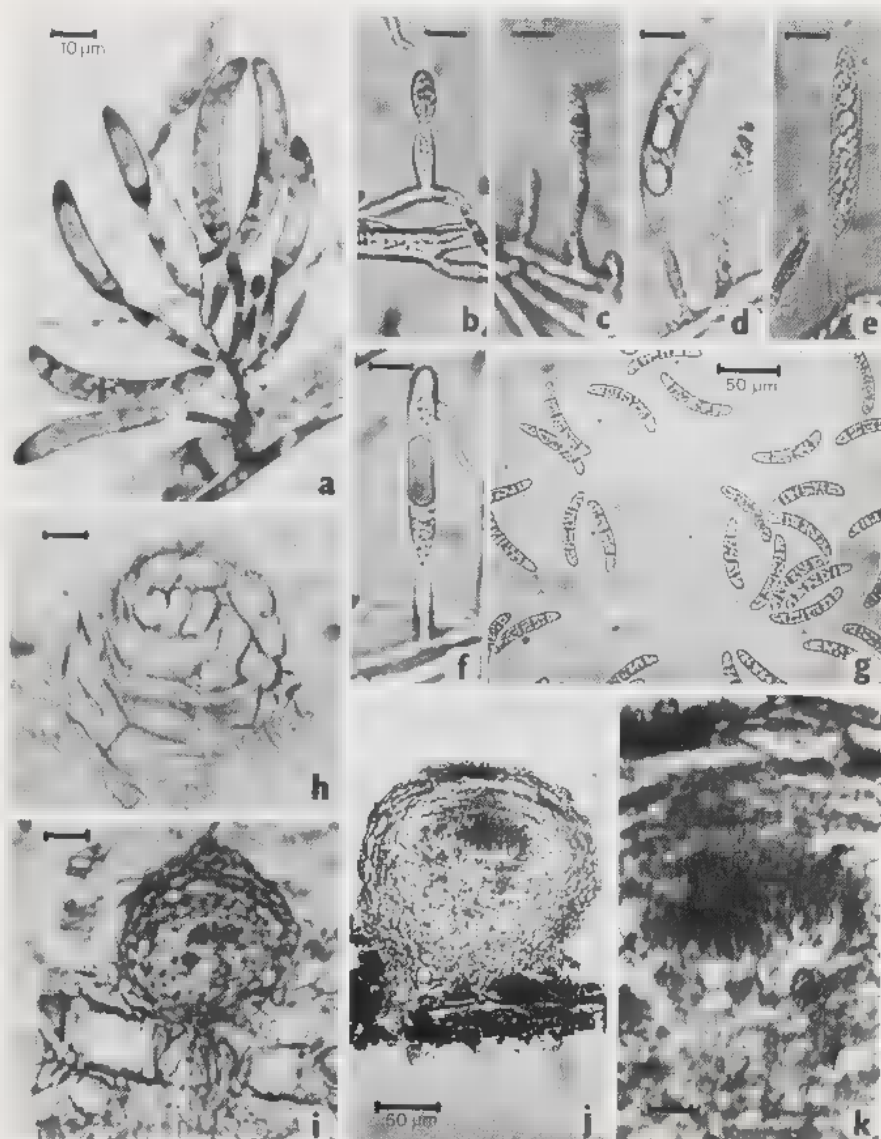


Plate I. — *Nectria kera* : a. a conidiophore with phialides and conidia; b-f. stages in the development of conidium; g. conidia; h. an ascogonium surrounded by hyphae; i. section through a coiled ascogonium surrounded by hyphae; j-k. sections through perithecial centrum showing apical paraphyses.

Planche I. — *Nectria kera* : a. conidiophore avec phialides et conidies; b-f. stades de développement d'une conidie; g. conidies; h. ascogone entouré d'hyphes; i. coupe à travers un ascogone enroulé, entouré d'hyphes; j-k. coupe à travers un jeune périthèce montrant des paraphyses apicales.

distinct verrucosities in the epispore, and the absence of microconidia. The fungus was isolated from spathe of *Cocos nucifera*. When inoculated on same substrate mature perithecia are formed after six weeks.

The possession of red or semi-translucent perithecia, and thick-walled, pseudoparenchymatous perithecial wall are features of the *Coccinea*-group of the genus *Nectria* (BOOTH, 1959). Compared to the five species so far known in the *Coccinea*-group (*Nectria coccinea* (Pers.) Fr., *N. galligena* Bres., *N. ditissima* Tul., *N. punicea* (Schmidt ex Fr.) Fr. ex Rabenh., *N. hederæ* Booth and *N. fockeliana* Booth) the perithecia and the ascospores are larger in our fungus and the latter being distinctly verrucose. Mostly species of *Nectria* in the *Coccinea*-group occur on hosts belonging to either dicots or Gymnosperms. Our fungus is isolated from a monocot (palm). There is no other taxon which combines the unique features of our fungus and we are therefore accommodating it in the genus *Nectria* as ■ new species.

#### DEVELOPMENT OF THE ANAMORPH

In slide culture macroconidia develop in 5-6 days after inoculation. Conidiophores arise as lateral branches on the vegetative hyphae. Phialides may be the terminal cells of the conidiophores or may arise laterally on the conidiophore. During the formation of a lateral phialide, a lateral bud arises on the conidiophore and elongates (Fig. 2). When it attains its full size, ■ basal septum delimits it from the conidiophore. The wall of the phialide is uniform in thickness except at the neck where it is slightly thickened.

The development of the conidium is as follows. Initially, the phialide is cylindrical in shape and rounded at the tip. Early in the development of the conidium, the tip of the phialide buds out a small protuberance (Fig. 3). With further development, the protuberance elongates and swells (Fig. 4-6; Plate I, b-c). As the conidium initial elongates its contents become granular and the conidium initial slightly bends in the middle (Plate I, d-f). When the conidium becomes fully mature, a septum is laid down in the neck region of the phialide delimiting the conidium (Fig. 7). The liberated conidium is smoothly rounded (Fig. 11, 12). As the first conidium is liberated, ■ second conidium initial appears in the open end of the phialide and develops into a second conidium (Fig. 9). This process is repeated so that ■ number of conidia are produced from ■ phialide in a basipetal sequence.

The chlamydospores usually develop in old cultures. The development of the chlamydospore is as follows (Fig. 13). The apical part of the terminal cell of a vegetative hypha gradually swells and is delimited from a subtending cell by the formation of a septum. The swollen apical cell is the developing chlamydospore and the contents of this cell and the subtending cell are granular. Mature chlamydospores are globose, thick-walled and contain dense cytoplasm.

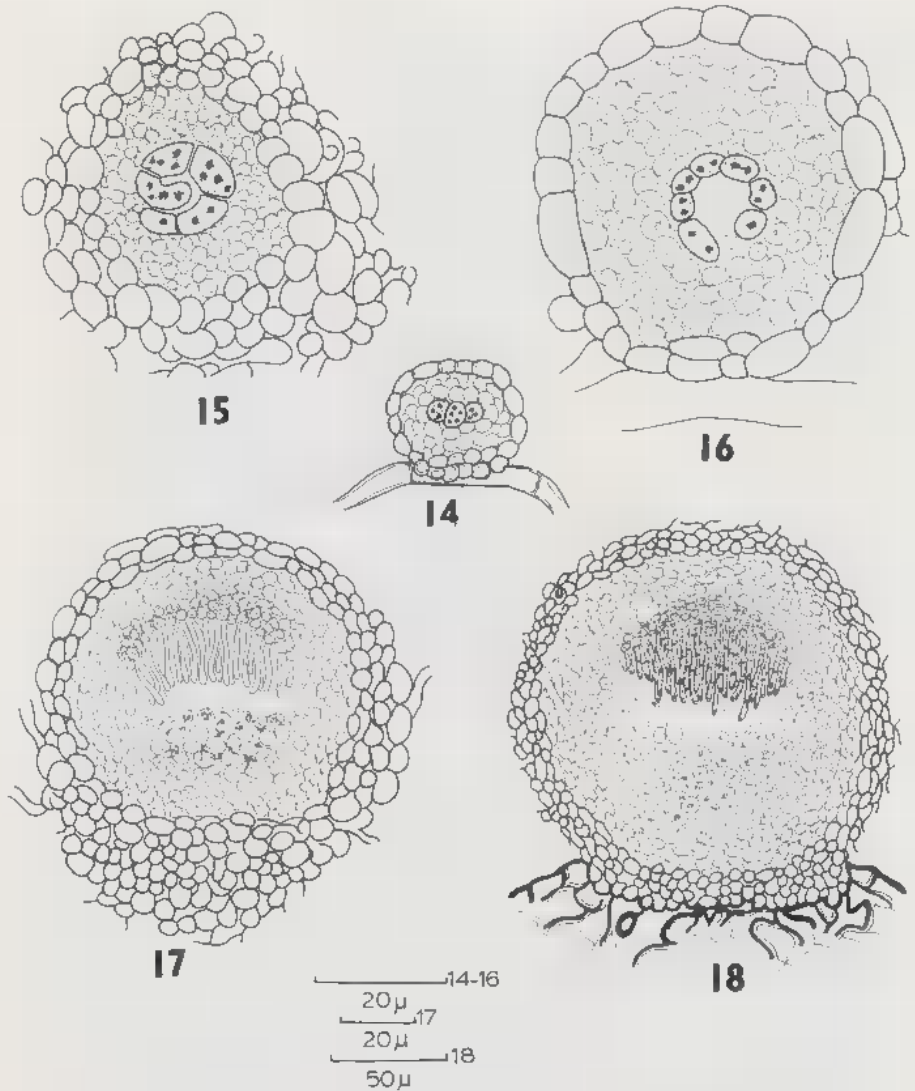
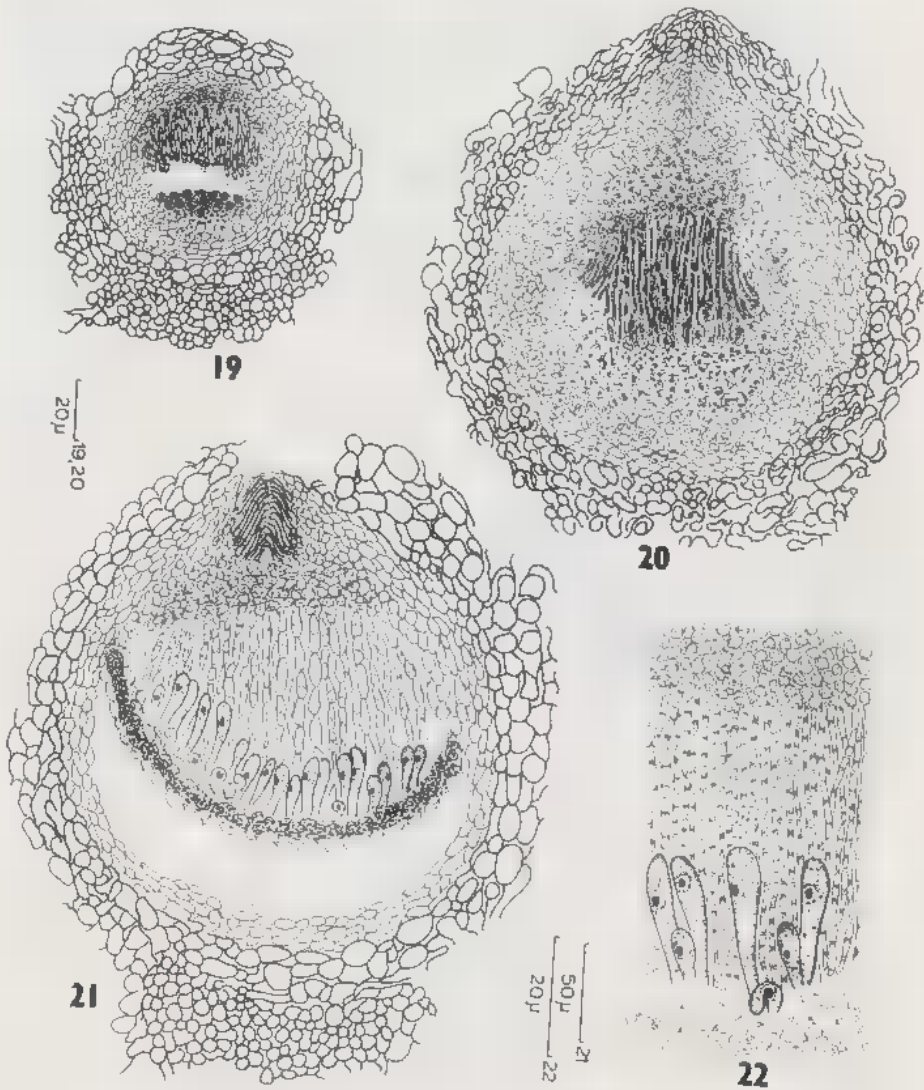


Fig. 14-18. — *Nectria kera*: 14-16, stages in the development of ascogonium and young perithecial centrum; 17-18, sections through young perithecial centra showing apical paraphyses.

Fig. 14-18. — *Nectria kera*: 14-16, développement d'un ascogone et d'un primordium de périthèce; 17-18, coupes à travers de jeunes périthèces montrant des paraphyses apicales.

Fig. 19-22. — *Nectria kera*: 19, section of perithecial centrum showing apical paraphyses. Note the well differentiated inner and outer regions of perithecial wall; 20, longitudinal





section of a perithecium showing apical paraphyses filling the entire centrum cavity; 21. section of perithecium showing the development of asci interspersed with apical paraphyses. Note also formation of ostiole lined with paraphyses; 22. portion of centrum showing asci interspersed with apical paraphyses (enlarged).

Fig. 19-22. — *Nectria kera* : coupes à travers des périthèces. 19. paraphyses apicales. On remarque la différenciation d'une couche interne et d'une couche externe dans la paroi du périthèce; 20. paraphyses apicales remplissant la cavité centrale; 21. développement des asques mêlés aux paraphyses apicales. On note la formation d'un ostiole bordé de paraphyses; 22. détail de 21.

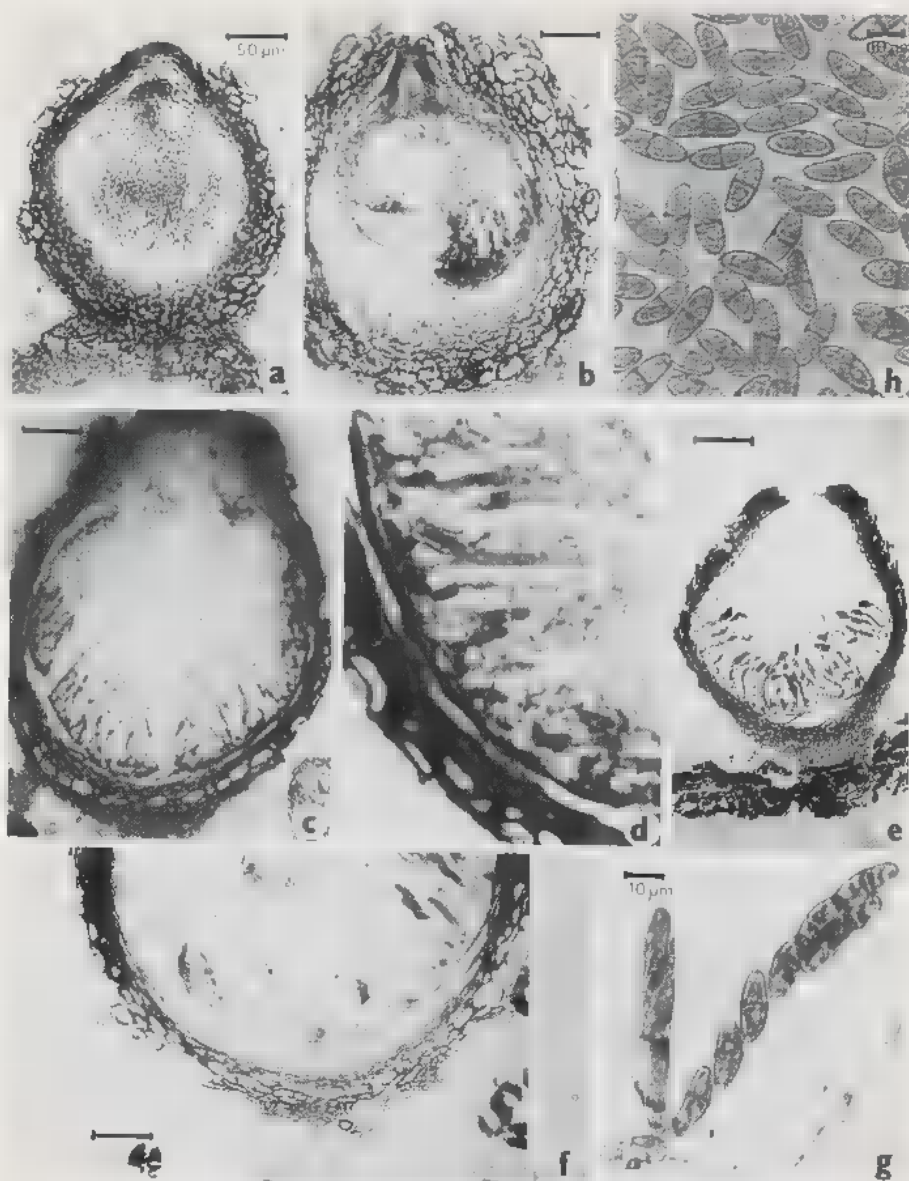


Plate II. — *Nectria kera* : a. section through a perithecial centrum showing apical paraphyses in the form of a palisade-like layer; b-d. longitudinal sections of young perithecia showing apical paraphyses (d = portion of c, enlarged); e-f. longitudinal sections of mature perithecia; g. asci; h. ascospores.

Planche II. — *Nectria kera* : a. coupe à travers un jeune périthèce montrant des paraphyses apicales groupées en palissade; b-d. coupes longitudinales d'un jeune périthèce montrant des paraphyses apicales (d = détail de c); e-f. coupes longitudinales de périthèces mûrs; g. asques; h. ascospores.



## DEVELOPMENT OF THE TELEOMORPH

The first indication of perithecial development observed by us is the appearance of an ascogonium containing 4-5 cells surrounded by thick-walled vegetative hyphae presumably developed from the base of the ascogonium (Plate I, h). A fully developed ascogonium is a coil of swollen multinucleate cells whose cytoplasm is dense and more deeply staining than the surrounding hyphal cells (Fig. 16). Each cell of the ascogonial coil contains 2-6 nuclei (Fig. 14). The cells of the hyphae surrounding the ascogonium now divide to produce a pseudoparenchymatous envelope of about 4-6 layers of cells of which the outer most layer becomes slightly thick-walled and inner cells remain thin-walled (Fig. 15; Plate I, i). At this stage the ascogonium becomes coiled and its cells become swollen and multinucleate. The thin-walled cells surrounding the ascogonium continue to divide and later differentiate into an inner zone of thin-walled pseudoparenchymatous cells and an outer zone of somewhat larger thick-walled pseudoparenchymatous cells and these two zones together constitute the wall of young perithecium (Fig. 17). In further development, a cavity is formed around the ascogonium presumably due to disintegration of cells in that region. At the apex of the cavity so formed, the thin-walled cells become «meristematic» and produce a palisade of darkly staining, thin-walled, septate, cylindrical filaments which grow down into the cavity (Fig. 18, 19; Plate I, j-k). These are the apical paraphyses. Cells of the apical paraphyses are uni- or binucleate.

During the downward growth of the apical paraphyses, the ascogonial cells are pushed down and the apical paraphyses ultimately touch the base of the perithecial cavity (Fig. 20; Plate II, a). The cells immediately below the ascogonium become enlarged and vacuolated. Asci are produced at the base of the perithecial cavity and grow upward interspersed with apical paraphyses (Fig. 21, 22; Plate II, b-d). Apical paraphyses eventually disintegrate (Fig. 23; Plate II, c) and mature perithecium is aparaphysate (Fig. 24; Plate II, e).

The formation of the ostiole takes place at the time the asci start growing upward (Fig. 20, 21; Plate II, a-b). Cells of the inner region of the perithecial wall at the morphological apex of the perithecium grow upwards and develop an ostiolar neck. By dissolution of cells in its core a narrow canal develops. The ostiolar canal extends from the centrum cavity to the exterior. The cells in the ostiolar neck are thick-walled and are in the form of unbranched hyphae with rounded tips (Fig. 25). The cells lining the ostiolar canal produce slender paraphyses.

## DISCUSSION

The anamorph of *Nectria kera* is a *Cylindrocarpon*-state. The first step in the development of the perithecium is the formation of a coiled, septate ascogonium, the cells of which are multinucleate. The perithecial centrum belong



Fig. 23-27. — *Nectria kera* : 23. longitudinal section of a young perithecium; 24. longitudinal section of a mature perithecium; 25. longitudinal section of ostiolar region (enlarged); 26. three asci and group of eight ascospores; 27. ascospores.

Fig. 23-27. — *Nectria kera* : 23. coupe longitudinale d'un jeune périthèce; 24. coupe longitudinale d'un périthèce mûr; 25. détail de la région ostiolaire; 26. asques et ascospores; 27. ascospores (détail).

to the *Nectria*-type (LUTTRELL, 1951) with apical paraphyses. Thus *Nectria kera* is a good hypocreaceous fungus. We have so far informations on the developmental morphology of as many as nine species of the genus *Nectria*, and *N. kera* is in conformity with all those, including *N. cinnabarina* (Tode ex Fr.) Fr. which is the type species of the genus.

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